PB90-219668

Continuous-Flow Method for Measuring Effects of Chemicals on Soil Nitrification

(U.S.) Corvallis Environmental Research Lab., OR

1990

U.S. Department of Commerce
National Technical Information Service
NTIS

ERL-COR- 962J

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before comple

1. REPORT NO. EPA/600/J-90/008	2.	3. PB90-219668
4. TITLE AND SUBTITLE A continuous-flow method for measuring effects of chemicals on soil nitrification		5. REPORT DATE 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Albert N. Rhodes and Charles W. Hendricks		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Dept of Biology, USAF Academy; USEPA, ERL-Corvallis, OR		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS US Environmental Protection Agency Environmental Research Laboratory 200 SW 35th Street Corvallis, OR 97333		13. TYPE OF REPORT AND PERIOD COVERED Journal Article 14. SPONSORING AGENCY CODE EPA/600/2

15. SUPPLEMENTARY NOTES
1990 Toxicity Assessment: An International Journal 5:77-89.

16. ABSTRACT

Because the productivity of terrestrial ecosystems is directly related to microbial nutrient cycling, understanding the effects of chemical contaminants on soil microbial processes is important. This study examined the effects of two model chemicals - Roundup (glyphosate) and N-Serve (nitrapyrin) - on nitrifying organisms in static, perfusion, and continuous-flow culture systems. Experimental concentrations were approximately 1, 10, and 100 X the spot application rate. Both products were shown to inhibit nitrification in the treated soils. Roundup significantly reduced nitrification at 6.8 and 68 mg g^{-1} dry soil. N-Serve completely inhibited nitrification at levels greater than 42 μg^{-1} dry soil in all cultural methods.

In comparative studies with static batch and perfusion culture techniques, the continuous-flow system proved to be both reliable and useful in the culture of nitrifying bacteria. This method provides an alternative to traditional culture techniques in measuring chemical effects on microbial geochemical cycles and provides a new method for use in toxicity testing.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified 20. SECURITY CLASS (This page) Unclassified	21. NO. OF PAGES 15 22. PRICE A03

EPA Form 2220-1 (9-73)

EPA/600/J-90/008

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

A Continuous-Flow Method for Measuring Effects of Chemicals on Soil Nitrification

ALBERT N. RHODES* and CHARLES W. HENDRICKS†

U.S. Environmental Protection Agency,
Environmentral Research Laboratory—Corvallis,
200 S.W. 35th Street, Corvallis, Oregon 97333

ABSTRACT

Because the productivity of terrestrial ecosystems is directly related to microbial nutrient cycling, understanding the effects of chemical contaminants on soil microbial processes is important. This study examined the effects of two model chemicals—Roundup (glyphosate) and N-Serve (nitrapyrin)—on nitrifying organisms in static, perfusion, and continuous-flow culture systems. Experimental concentrations were approximately 1, 10, and 100× the spot application rate. Both N-Serve and Roundup were shown to inhibit nitrification in the treated soils. Roundup significantly reduced nitrification at 6.8 and 68 mg g^{-1} dry soil. N-Serve (nitrapyrin) completely inhibited nitrification at levels greater than 42 μ g^{-1} dry soil in all cultural methods.

In comparative studies with static batch and perfusion culture techniques, the continuous-flow system proved to be both reliable and useful in the culture of nitrifying bacteria. This method provides an alternative to traditional culture techniques in measuring chemical effects on microbial geochemical cycles and provides a new method for use in toxicity testing.

INTRODUCTION

The productivity of terrestrial ecosystems, as measured by the growth of higher plants, is controlled by the availability of essential inorganic nutrients. The microorganisms present in a soil, and the rates at which they can cycle these nutrients, largely determine the amount of nutrients available to plants. By metabolizing organic matter in soil, microorganisms can return nutrients in a plant-usable inorganic form. A healthy and active microbial community, therefore, is a prerequisite to soil fertility (Thompson and Troeh, 1978). However, many nutrient cycling pathways are mediated by only a few genera of bacteria (Atlas

* Present address: Department of Biology, U.S. Air Force Academy, Colorado Springs, Colorado 80840-5701.

† To whom correspondence should be directed.

Toxicity Assessment: An International Journal Vol. 5, 77–89 (1990)
Not subject to copyright within the United States.
Published by John Wiley & Sons, Inc. CCC 0884-8181/90/050077-013$04.00

and Bartha, 1987). So important are these bacteria to soil processes that if they were not able to function, higher terrestrial life would be endangered (Brady, 1974). Therefore, a disturbance or inhibition of a small segment of a soil microbial community, brought about by the application of a toxic xenobiotic compound, may critically affect soil productivity.

The biological oxidation of ammonium to nitrate in soil is facilitated primarily by two groups of chemolithotrophic bacteria: ammonium oxidizers and nitrite oxidizers. Inhibition of either of these two groups may significantly alter the dynamics and balance of the soil nitrogen pool. These organisms, however, grow very slowly and are difficult to maintain and study in pure culture. Consequently, most studies focus on the impact of a compound on nitrification rates. This approach, unfortunately, does not address the effects of a compound on the growth or death of nitrifying bacteria.

This study was designed with two objectives: (1) to evaluate the continuous-flow method with the traditional static and perfusion techniques used to study nitrification in soil, and (2) to determine the response of nitrifying and heterotrophic bacteria to herbicide treatment using the three different culture techniques. Preliminary experiments conducted as part of this study determined that of the three cultural methods, the continuous-flow method supported high nitrifier activity and was most sensitive to the action of nitrification inhibitors at low application rates. For this reason, the present study evaluated the continuous-flow method as a way of measuring the effects of chemical treatment on ammonium and nitrite oxidizing bacteria in soil.

METHODS AND MATERIALS

Soil

Initially, four different soils were screened for nitrifying bacterial activity. They were collected from the foothills of the California Sierra Nevada mountains, the Cascade Head Experimental Forest in the Oregon Coast Range, and two from different locations in the Willamette Valley, Benton County, Oregon. After these preliminary studies, only one of the Willamette Valley soils had nitrifying activity suitable for the evaluation of nitrification by the continuous-flow culture technique, and it was chosen for further study.

The soil used in the study is well characterized and employed routinely by the U.S. Environmental Protection Agency (EPA) for air quality research on plants. It is a fine, silty, mixed mesic Agriaquic Xeric Agialboll of the Amity series and is primarily used for agriculture

TABLE I
Chemical Analysis of Amity Soil[a]

Constituent	Value
Ca	14.6 meq 100 g^{-1}
K	1.9 meq 100 g^{-1}
Mg	3.36 meq 100 g^{-1}
NH_4	5.6 ppm
NO_3	173.6 ppm
Total-N	0.24%
Total-P	14.6 ppm
TOC[b]	3.47%
CEC[3]	22.6 meq 100 g^{-1}
pH	5.4

[a] Analyses performed by the Soils Testing Laboratory, Oregon State University, Corvallis, OR.
[b] TOC: total organic carbon.
[c] CEC: cation exchange capacity.

(Knezevich, 1975). Amity silty clay loams are typically deep, somewhat poorly drained, and were formed in mixed alluvium terraces. These soils receive an average of 110 cm annual rainfall, but are dry for most of the summer months. The chemical analysis of the soil was performed by the Soils Testing Laboratory, Oregon State University, Corvallis, Oregon (Table I).

Bulk soil samples were collected randomly from a depth of 0–4 cm. The soil was allowed to incubate at ambient laboratory temperature (25–27°C) for approximately 5 days, sieved to pass a 2-mm screen, and stored in plastic bags at 5 ± 2°C until use (Anderson and Domsch, 1979). This procedure was used to remove gravel, larger aggregates, plant debris, and to provide uniform particle size and biological activity for experimental use.

Nitrification Studies

Culture Techniques

Three techniques were used to examine the effects of chemicals on nitrification of Amity soil. These techniques were the continuous-flow method (Hendricks *et al.*, 1987), the perfusion column (Lees and Quastel, 1946), and the static batch culture (Alexander, 1961).

Continuous-Flow Culture. Soil columns were prepared from 47-mm

Nucleopore (Pleasanton, CA) filter holders* with a stacking adapter attached to provide sufficient volume for the addition of soil (the design and operation of this device is reported elsewhere; Hendricks *et al.*, 1987). This design allowed each column to hold 15.0 g dry soil with a soil column height of approximately 15 mm. The nitrification medium (250 μg NH_4-N mL^{-1}) was adjusted to pH 7.2 (Belser and Schmidt, 1982) and passed through the soil columns using an Cole Parmer 16-channel peristaltic pump (Cole-Parmer Instrument Co., Chicago, IL) that was calibrated to deliver 10.0 mL of growth medium day^{-1}.

Perfusion Culture. The soil perfusion apparatus for use in nitrification studies was first developed by Lees and Quastel (1946). This device permits circulation of growth medium through the soil for extended periods of time to support the growth of the desired microbial population and to extract metabolites from the soil. The apparatus used in this study was similar to the continuous-flow except that a line was added to permit recirculation of the nutrient medium. Other control parameters including the media, soil, and flow rates were the same as described for the continuous-flow technique above, but additional substrate was not added to the perfusion system.

Static Culture. In the static batch studies, 15.0 g of equivalent air dry soil was incubated in 100-mL screw-capped containers and amended with 250 mg NH_4-N g^{-1} dry soil. Distilled water was added to each culture to raise the final moisture content of the soil to 55% field capacity, and containers were opened daily to the atmosphere to provide for aeration.

Cultural Conditions. Because of the poor structure of the Amity soil, it was necessary to aerate both the continuous-flow and perfusion columns with bottled compressed air. This modification prevented the columns from flooding by forcing excess media through the soil and ensuring that aerobic conditions were maintained. Under the conditions described for this study, columns were aerated at a rate of 50 mL compressed air min^{-1}. The static cultures were allowed to aerate by opening the culture container once every two days. The incubation temperature for all culture techniques was 25 ± 1°C.

Chemical Treatments

In separate experiments, N-Serve 24 (nitrapyrin) (Dow Chemical Co., Midland, MI) and Roundup (glyphosate) (Monsanto Agricultural Prod-

* Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ucts Co., St. Louis, MO) were applied to triplicate soil cultures, and the production of nitrate followed through the course of the experiment. Both N-Serve and Roundup were obtained in commercial formulation with a stated label concentration of 240 and 397 g active ingredient (AI) L^{-1} stock solution, respectively. When necessary, dilutions of the commercial formulation were prepared in distilled water. Since N-Serve 24 is not water soluble, aqueous dilutions were homogenized with a vortex mixer for 1 min prior to addition to the soil. When added to the soil, the dilutions produced the following calculated concentrations: 0, 0.042, 0.42, and 4.2 mg AI g^{-1} dry soil for N-Serve and 0, 0.68, and 68 mg AI g^{-1} dry soil for Roundup.

Static and perfusion cultures were treated on day 0, but to ensure an adequate nitrifying population in the continous-flow columns, treatment was done on day 8. All cultures were sampled in duplicate and frozen at −15°C for later chemical analysis at the end of an experiment.

Chemical Analysis

Prior to analysis, the static soil cultures were extracted with 30 mL of 0.5 M K_2SO_4 for 30 min (Brookes *et al.*, 1985) on a rotary shaker (New Brunswick Scientific, New Brunswick, NJ) at 150 rpm and then followed by gravity filtration through Whatman No. 40 filter paper. Extraction was not necessary for the perfusion and continuous flow samples, and the eluates were analyzed directly without further preparation.

Analyses of samples for NH_4, NO_2, and NO_3 were performed with Technicon Autoanalyzer II (Technicon Instrument Co., Tarrytown, NY) using standard EPA chemical methods (U.S. EPA, 1979). Using these procedures, detection levels were 0.005 mg L^{-1} for NO_3 and NO_2, and 0.01 mg L^{-1} NH_4. When necessary, dilutions of the soil extracts were prepared with distilled/deionized water using a Fischer Dilumat automatic dilutor (Fischer Scientific Corp., Pittsburgh, PA). The dilutors were calibrated to perform 5- and 20-fold dilutions, and set to deliver 5 mL working volumes, ±0.16 mL (S^2 = 0.20) and ±0.03 mL (S^2 = 0.01), respectively.

All pH measurements were obtained using an Orion Model 901 Ionalyzer fitted with an Orion 91-15 gel-filled semi-micro combination electrode (Orion Research Inc., Cambridge, MA) and calibrated against standard pH 4.0 and 7.0 buffers.

Statistical and Data Quality Considerations

All experiments were based upon a completely randomized design, and statistical differences between controls and treatments were deter-

mined using the analysis of variance statistic. The test of significance was at the $p < 0.01$ level.

The reported concentrations of NH_4, NO_2, and $NO_2 + NO_3$ were calculated automatically based upon the values from known chemical standards and method blanks. To ensure that the instrument was performing within laboratory quality assurance limits, every fifth sample was analyzed in duplicate. If the duplicate samples were not in agreement (within 5%), the samples were reanalyzed. Periodically, certified EPA standard samples were analyzed to verify instrument calibration.

RESULTS

The nitrification in Amity soil occurring over an 18-day incubation period is presented in Fig. 1. The cumulative production of nitrate in static, perfusion, and continuous-flow methods are plotted vs time. Data represent concentrations of nitrate in soil extracts or column effluents compiled using controls from each of the three methods. Slopes of the lines for each of the three techniques are representative of the rate of nitrification over the incubation period. These rates are not directly comparable because the kinetics of substrate addition and waste removal vary with the culture technique. The amount of nitrate produced

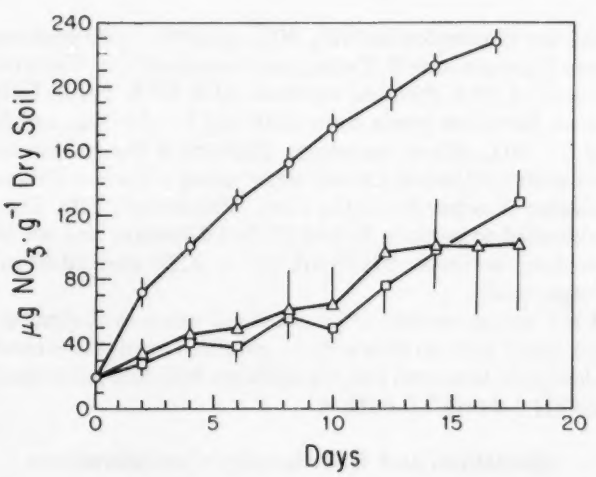

Fig. 1. Nitrification in soil using static (△), perfusion (□), and continuous-flow (○) culturing techniques.

was greatest using the continuous-flow method, but differences in nitrate production in the static and perfusion studies were not statistically significant.

The results of nitrapyrin (N-Serve) soil treatments are shown in Fig. 2. In static batch cultures, nitrification was significantly inhibited at 0.042, 0.42, and 4.2 mg nitrapyrin g^{-1} dry soil by day 16 (Fig. 2A). Although significantly less than in the untreated controls ($p < 0.01$), nitrification was observed in the 0.042 mg nitrapyrin g^{-1} dry soil treatments. Complete inhibition was obtained at 0.42 and 4.2 mg nitrapyrin g^{-1} dry soil, as demonstrated by the relatively constant levels of nitrate in the soil over the course of the experiment. In all cases, only trace levels of nitrite were detected in the soil.

Data obtained from the nitrapyrin treatment using the perfusion columns are presented in Fig. 2B. Untreated controls nitrified slightly over the 11 days these columns were incubated. A decline in nitrate is evident toward the end of the experiment. By the end of the incubation period, nitrification was significantly inhibited in at all treatment levels. Loss of NH_3 was observed in the columns receiving 4.2 mg nitrapyrin g^{-1} dry soil.

Inhibition of nitrification was complete at all treatment levels in the continuous-flow columns (Fig. 2C). Untreated controls actively nitrified throughout the 16-day incubation period. Samples were treated with nitrapyrin on day 8, and nitrate production ceased after day 10 at all concentrations.

The effect of glyphosate (Roundup) treatment in the static cultures is shown in Fig. 3. Levels of Roundup at 0.68 and 6.8 mg glyphosate g^{-1} dry soil reduced nitrification in static batch cultures (Fig. 3A). Soil treated with 68 mg glyphosate g^{-1} dry soil was saturated, lost structure, and was not further examined. Although nitrification was evident in samples treated with 0.68 and 6.8 mg glyphosate g^{-1} dry soil, the activity of nitrifying bacteria was low. In these cultures, high concentrations of Roundup tended to disperse the soil aggregates.

Figure 3B illustrates the response of the nitrifying bacteria to Roundup treatment in perfusion columns. The effects, as measured by the slope of the treatment line, indicate that nitrification occurred in the controls, and at 0.68 and 6.8 mg glyphosate g^{-1} dry soil. However, there was no statistically significant difference between the controls and the three treatments.

Soil treated with glyphosate in the continuous-flow device demonstrated inhibition of nitrification at the two highest treatment levels (Fig. 3C). Differences between the controls and those columns receiving 6.8 and 68 mg glyphosate g^{-1} were highly significant ($p < 0.01$) on day 16. Although the soils receiving 0.68 mg glyphosate g^{-1} dry soil appear

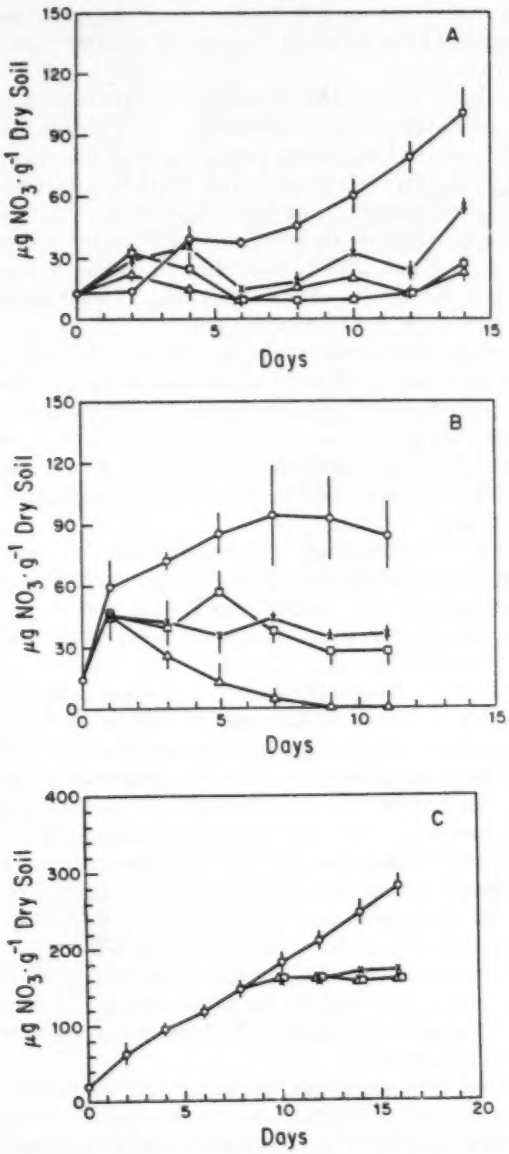

Fig. 2. Effects of N-Serve on soil nitrification in static (A), perfusion (B), and continuous-flow (C) culture. N-Serve concentration: 0 (○), 0.042 (×), 0.42 (□), 4.2 (△) mg nitrapyrin g^{-1} dry soil.

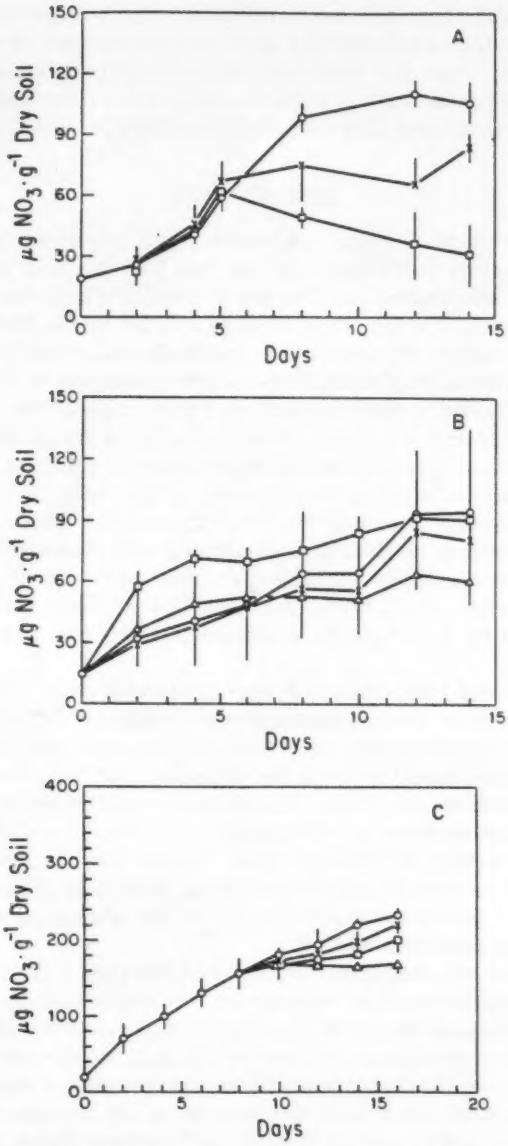

Fig. 3. Effects of Roundup on soil nitrification in static (A), perfusion (B), and continuous-flow (C) culture. Roundup concentration: 0 (○), 0.68 (×), 6.8 (□), and 68 (△) mg glyphosate g^{-1} dry soil.

to be inhibited, they were not significantly different from the controls. Nitrification increased after day 14 in the columns treated with 6.8 mg glyphosate g^{-1} dry soil. Effluents from the columns treated with 6.8 and 68 mg glyphosate g^{-1} dry soil were noticeably colored (dark brown) 2 days after treatment. The coloration decreased in intensity over time.

DISCUSSION

The routine study of chemolithotrophic nitrifying bacteria is difficult under laboratory conditions and has resulted in few studies on the ecology of these organisms. The use of nitrifying bacteria for toxicity measurements, however, should not be avoided due to the limitations of culture methods. An objective of this study was to examine an alternative to existing culture methods. A new continuous-flow soil culture method, first used to study respiration and biomass of soil microorganisms, was modified to culture nitrifying bacteria in soil. This continuous-flow method is unique and differs from previous methods by adding substrate to a soil column continuously at low rates. Column eluates are collected simultaneously for chemical analysis. Soils can be maintained at moisture levels near field capacity while substrate concentrations remain relatively unchanged throughout the incubation period (Hendricks *et al.*, 1987). With minor modification, soil moisture content at any desired level can be maintained using the continuous-flow system.

To determine the efficacy of a new method to study nitrification in soil, it is necessary to evaluate the procedure against currently accepted techniques. In this study, static batch cultures and soil perfusion columns, two traditional methods for culturing nitrifying bacteria, were compared with the continuous-flow system. A major limitation of batch and perfusion systems is their constantly changing soil chemistry in laboratory culture. In addition, both of these systems are closed, and are difficult to monitor and adjust during incubation. The continuous-flow method overcomes this limitation by the addition of substrate at a constant predetermined rate.

To effectively maintain soil bacterial biomass within the column, fine soil particles must be retained and not washed out by the flowing medium. Throughout the study, eluates demonstrated no observable turbidity or accumulation of sediments within the effluent tubing. We believe the loss of fine soil particles would be a serious shortcoming in this or any other continuous-flow design, as the activity of the soil is dependent on organisms attaching to soil surfaces (Keen and Prosser, 1988; Underhill and Prosser, 1987).

Early in this study it was also necessary to modify the continuous-

flow columns to allow for soil aeration. Soil structure was reduced by sieving, thereby preventing infiltration of medium into the soil and resulted in column flooding. Analysis of eluates from flooded columns indicated that nitrate production was drastically reduced, but this observation was not further investigated. Using forced aeration it was possible to keep the soil pores open and prevent the soil from flooding.

From these initial studies, data were collected to determine nitrifier activity in the three systems. Figures 2 and 3 indicate that the activity in continuous-flow columns was higher than in static and perfusion methods. The continuous-flow system demonstrated higher levels of nitrifier activity due to constant concentrations of oxygen and substrate. This removes errors associated with soil extraction and sampling from within the perfusion reservoir. Additionally, the medium is added and eluted at a constant rate, which allows the soil within the column to reach equilibrium. In these experiments, nitrite was not observed and ammonia was always in excess.

Since most natural soil environments are for the most part at steady-state equilibrium for only short periods of time (Bohn *et al.*, 1979), the use of the continuous-flow method to control soil parameters in laboratory experiments is clearly advantageous in studying the ecology of soil microorganisms. This level of control of the chemical and physical properties of a soil cannot be achieved using either the static or perfusion systems, which is a drawback to their operation and use in the study of the effects of chemicals on microbial geochemical processes.

Bacterial growth was observed along the walls of the tubing carrying medium into the perfusion columns. This growth may have introduced unwanted activity into the experiment. For example, loss of nitrate was observed in perfusion columns treated with 4.2 mg nitrapyrin g^{-1} dry soil. This observation was not seen in the other two methods at the same treatment level (Figs. 2 and 3). The most plausible explanation for this observation is the combination of growth of bacteria along the walls of the pump tubing at reduced oxygen levels and the potential anaerobic microsites within the soil matrix (Parkin, 1987).

Chemical treatment studies with N-Serve and Roundup focused on the response of nitrifying bacteria native to Amity soil used in this study. As a known nitrification inhibitor, N-Serve was used as a positive control on inhibition of a nitrifying population by a chemical substance. Nitrification was inhibited significantly by N-Serve, at all levels, in all three methods.

A comparison of the three methods (Figs. 2 and 3) reveals that nitrapyrin inhibition is most readily determined using the continuous-flow method. In the other two methods, the effects may be misleading due to the variation in samples. The wide variation in samples collected

from static incubations could be attributed to the natural variation within soil cultures. The variation in the perfusion studies cannot be explained in the same way as sample variability should have paralleled the variation exhibited in the continuous-flow columns.

The response to N-Serve treatment appears to be dose dependent, although all concentrations significantly inhibited nitrification. The dose response can be seen in the static and perfusion culture data, but because of the design of the continuous-flow system, concentrations above those causing complete inhibition cannot be resolved. Since the activity of the nitrifying bacteria was highest in the continuous-flow columns, the organisms may be more sensitive to the action of chemical inhibitors (Powell and Prosser, 1986). Sensitivity, however, varies greatly among the genera of nitrifying bacteria (Belser and Schmidt, 1981; Gadkari, 1988).

Glyphosate did not inhibit nitrifying bacteria in Amity soil at levels approaching field application rates. Previous studies (Carlisle and Trevors, 1986) have shown Roundup to be inhibitory at 0.77 mg glyphosate g^{-1} dry soil. This study has shown that Roundup is not inhibitory at 0.68 mg glyphosate g^{-1} dry soil, but does exhibit intermediate and complete inhibitory effects at 6.8 and 68 mg, respectively. This observation indicates that, although Roundup can be a potential inhibitor of certain microbial activities in pure culture (Bode *et al.*, 1985; Fischer *et al.*, 1986), inhibition did not occur with the natural populations used in this study at recommended application levels of the herbicide.

High levels of Roundup disrupted soil aggregates in treated columns. Dispersion appeared to be greatest in static cultures where soils lost nearly all of their structure. Effects were not as pronounced in perfusion and continuous-flow soils as in treated static cultures. The constant flow of media containing the herbicide with these two methods may have prevented dispersion.

The continuous-flow method for culturing nitrifying bacteria has proven to be a viable alternative to existing methods to culture these bacteria. In this study, the continuous-flow system was used in nitrification studies to determine the impact of chemical compounds on a natural soil process. The use of this continuous-flow method in this and previous experiments (Hendricks *et al.*, 1987) indicates that it has potential for use in toxicity assessment and other areas of microbial ecology.

References

Anderson, J.P.E., and K.H. Domsch. 1979. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol. Biochem. *10*:215–221.

Alexander, M. 1961. Introduction to Soil Microbiology. John Wiley & Sons, New York. 472 p.

Atlas, R.M. and R. Bartha. 1987. Microbial Ecology: Fundamentals and Applications (2nd ed.). Benjamin/Cummings Publishing Company, Menlo Park, CA.

Belser, L.W., and E.L. Schmidt. 1981. Inhibitory effect of nitrapyrin on three genera of ammonia-oxidizing nitrifiers. Appl. Environ. Microbiol. *41*:819–821.

Bode, R., G. Kunze, and D. Birnbow. 1985. Reversal of glyphosate-induced growth inhibition of *Candida maltosa* by several amino acids and pyruvate. Biochem. Physiol. Pflanzen. *180*:613–619.

Bohn, H.L., B.L. McNeal, and G.A. O'Connor. 1979. Soil Chemistry. John Wiley & Sons, New York.

Brady, N.C. 1974. The Nature and Properties of Soils (8th ed.). Macmillan Publishing Company, New York, 639 p.

Brookes, P.C., A. Landman, G. Pruden, and D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. *17*:837–842.

Carlisle, S.M., and J.T. Trevors. 1986. Effects of the herbicide glyphosate on nitrification, denitrification, and acetyl reduction in soil. Water Air Soil Pollut. *29*:189–203.

Fischer, R.S., A. Berry, C.G. Gaines, and R.A. Jenson. 1986. Comparative action of glyphosate as a trigger of energy drain in *Eubacteria*. J. Bacteriol. *168*:1147–1154.

Gadkari, D. 1988. Effects of atrazine and paraquat on nitrifying bacteria. Arch. Environ. Contam. Toxicol. *17*:443–447.

Hendricks, C.W., E.A. Paul, and P.D. Brooks. 1987. Growth measurements of terrestrial microbial species by a continuous-flow technique. Plant and Soil *101*:181–195.

Keen, G.A., and J.I. Prosser. 1988. The surface growth and activity of *Nitrobacter*. Microb. Ecol. *15*:21–39.

Knezevich, C.A. 1975. Soil Survey of Benton County Area, Oregon. U.S. Department of Agriculture, Soil Conservation Service, Washington, D.C. 119 p.

Lees, H., and J.H. Quastel. 1946. Biochemistry of nitrification in soil. 2. The site of soil nitrification. Biochem. J. *40*:803–815.

Parkin, T.B. 1987. Soil microsites as a source of denitrification variability. Soil Sci. Soc. Am. J. *51*:1194–1199.

Powell, S.J., and J.I. Prosser. 1986. Inhibition of ammonium oxidation by nitrapyrin in soil and liquid culture. Appl. Environ. Microbiol. *52*:782–787.

Thompson, L.M., and F.R. Troeh. 1978. Soils and Soil Fertility. McGraw-Hill, New York. 516 p.

Underhill, S.E., and J.I. Prosser. 1987. Surface attachment of nitrifying bacteria and their inhibition by potassium ethyl xanthate. Microb. Ecol. *14*:129–139.

U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. EPA Report EPA-600/4-79-020, Environmental Monitoring and Sampling Laboratory, Cincinnati, OH.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.